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Supplemental material

Supplement 1. Drug therapy with memantine in three patients with gain-of-function variants

Results of therapy data

Patient p.(Asn615Ile), aged 6 years and 6 months, displayed severe ID and recurrent series of epileptic spasms twice per week as well as tonic-clonic seizures once per week under treatment with Lamotrigine before starting memantine treatment. After four months of memantine treatment with 9 mg (0.6 mg/kg/day), EEG showed improvement of background rhythm from 6 Hz to 7-8 Hz. Frequency of spasms was unchanged, but the child showed improvement in awareness according to the parents. After additionally initiating ethosuximide due to the persistence of tonic-clonic seizures, therapy with memantine and ethosuximide was stopped after 10 months. Thereafter, the EEG background rhythm changed back to about 6 Hz. The child showed further improvement of awareness compared to the period of treatment with memantine and ethosuximide. Seizure frequency did not change significantly before, during and after treatment with memantine.

Patient p.(Val618Gly), aged 3 years and 3 months, displayed severe ID but was free of epileptic spasms for several months. Therapy with valproate was continued before initiating memantine. After 4 months of memantine treatment with 0.5 mg/kg/day, the child showed improvement in awareness and reduced restlessness according to parents and kindergarten teacher. Valproate therapy was discontinued due to normal EEG and absence of seizures/spasms. After 16 months of memantine treatment, the patient continued to show improved awareness and improved exploration with his hands according to controlled video documentation. After tapering of memantine medication, the patient showed reduced awareness, reduced eye contact and increased restlessness, so that memantine medication was started again. Thereupon, the patient again showed a noticeable improvement in awareness and reduced restlessness.

Patient p.(Gly611Val), aged 8 years, displayed severe ID and generalized seizures. After four months of memantine treatment (0.5 mg/kg/day), the patient showed slight improvements in walking and awareness according to the neuropediatrician. Parents reported improvements in awareness, social interaction and sleep pattern with reduced nightly arousals, but seizure frequency remained unchanged.

Patient p.(Met818Thr), aged 21 months, developmental delay, focal- and generalized seizures as well as cortical visual impairment. Treatment with memantine (0.5 mg/kg/day) was added to topiramate and after a period of five months the child's awareness was improved according to parents. Seizure frequency was unchanged and EEG showed deterioration due to novel multifocal epileptic foci.

Discussion of therapy data

Prompted by the successful treatment of a patient with *GRIN2A* encephalopathy with memantine[1], we evaluated memantine responses of four patients with (likely) pathogenic gain-of-function variants in *GRIN2B*. All four patients showed initial improvement of awareness according to parents. Improvement in awareness in patient p.(Asn615Ile) could not be validated long-term and as the frequency of epileptic spasms was not reduced, memantine treatment was stopped. So far, long-term follow-up data is available in only one patient p.(Val618Gly), who showed improvement in awareness and hand exploration. However, it remains unclear whether these improvements were due to memantine treatment or corresponded to the natural developmental course. Further patients and treatment data is needed to determine the potential therapeutic benefit of NMDAR blockers in a *GRIN2B* encephalopathy with double blinded prospective clinical trials with a relatively homogeneous patient population in terms of variant class.

Supplement 2. Variants of unknown significance (VUS)

We detected one missense variant c.1598G>A, p.(Gly533Asp) in a patient with moderate intellectual disability (ID), focal and generalized seizures and signs of generalized volumes on cMRI, a phenotype compatible with the described spectrum of *GRIN2B* encephalopathy. A *de novo* origin of the variant could not be proven as a paternal blood sample was not available. This variant is absent in the mother and in ExAC controls and the respective amino acid is highly conserved across 15 species up to C. elegans. Multiple *in-silico* prediction tools such as MutationTaster and PolyPhen predict a deleterious effect of the variant on GluN2B. According to established guidelines for the interpretation of sequence variants, this variant lacked sufficient criteria for likely pathogenicity and was therefore classified as a VUS[2]. Another *de novo* missense variant p.(Arg1111His) and a *de novo* inframe deletion p.(Lys976del) are located in the carboxy-terminal domain (CTD). Both variants were classified as VUS, as in ExAC considerably more missense variants are listed in the CTD compared to ligand-binding and transmembrane domains.

Two truncating variants in the CTD [p.(Ile864Ser*fs**20) and p.(Tyr1004*)] in patients with ID establish premature termination codons in the last exon. Premature termination codons located either in the last exon or within 50-55 nucleotides upstream of the 3'-most exon-exon junction likely fail to elicit NMD and both variants would therefore likely escape nonsense-mediated mRNA decay and possibly lead to a functional protein[2, 3]. We thus classified both variants as VUS. Our data does not allow the definition of a *GRIN2B*-specific cutoff position for pathogenicity/haploinsufficiency of truncating variants without additional functional analyses.

Supplement 3. Enrichment of *de novo GRIN2B* variants in combined WES trio cohorts

We aimed to test for enrichment of *de novo* missense variants in *GRIN2B* in different data sets of WES trios of patients with neurodevelopmental disorders. We used a Poisson test to compare the expected mutation rate of *GRIN2B* missense variants as previously described[4] with the rate of observed *GRIN2B* variants in patient cohorts. We tested data from three commercial diagnostic laboratories as well as the cohort of the Deciphering Developmental Delay Study (DDD). [5–8] Cohort sizes were 582, 767, 1082 and 4293 patients, respectively. The numbers of *de novo GRIN2B* missense variants were 2, 3, 4 and 7, respectively. A significance threshold robust to multiple testing was estimated with the Bonferroni method assuming that no more than 20,000 genes were tested in the mentioned WES trio studies. Testing the combined cohorts from diagnostic laboratories resulted in a p-value of $1x10^{-9}$ (below the multiple testing threshold of $5x10^{-7}$) which is in agreement with significant enrichment of *de novo GRIN2B* missense variants in the DDD cohort. Combining all four cohorts increased significance to a p-value of $2x10^{-17}$. *De novo* truncating variants (n=2) are nominally enriched in the combined cohort (p-value $1x10^{-4}$). This p-value was not significant after multiple testing corrections.

These analyses confirm the role of *de novo GRIN2B* missense variants in patients with neurodevelopmental disorders, but the sample size in available unselected cohorts may not be large enough for a final evaluation concerning the enrichment of *de novo* truncating variants.

	Current amplitude, nA_by 100 uM glutamate/100 uM glycine	Current amplitude, nA_by 1000 uM glutamate/100 uM glycine
WT 2B	650 ± 146 (14)	695 ± 160 (14)
S34Qfs*	5.2 ± 1.3 (13)	5.2 ± 2.1 (6)
Q180*	17 ± 4.1 (13)	9.6 ± 2.3 (8)
W559*	9.2 ± 4.0 (6)	17 ± 6.3 (6)
A636P	8.4 ± 2.1 (8)	13 ± 3.3 (8)
Q656*	13 ± 5.2 (17)	10 ± 5.8 (10)
G820E	7.4 ± 2.7 (28)	6.4 ± 2.0 (14)
M824R	5.8 ± 1.1 (25)	8.4 ± 1.9 (14)

Supplemental Table 1. Summary of current amplitude

Mean ± SEM (n), holding at -40 mV

	IC_{50}, µM (n)	maximal inhibition, %[#]	n
WT 2B	1.7 ± 0.2	40 ± 2.3%	26
G611V	1.0 ± 0.1	50 ± 2.0%	6
N615I	0.44 ± 0.05*	68 ± 2.1%*	6
V618G	16 ± 0.8*	7.0 ± 1.5%*	6
S810R	4.3 ± 0.6*	24 ± 2.6%*	5
M818T	3.0 ± 0.5*	25 ± 3.6%*	9
A819T	5.0 ± 0.7*	18 ± 2.9%*	5

Supplemental Table 2: Summary of memantine data

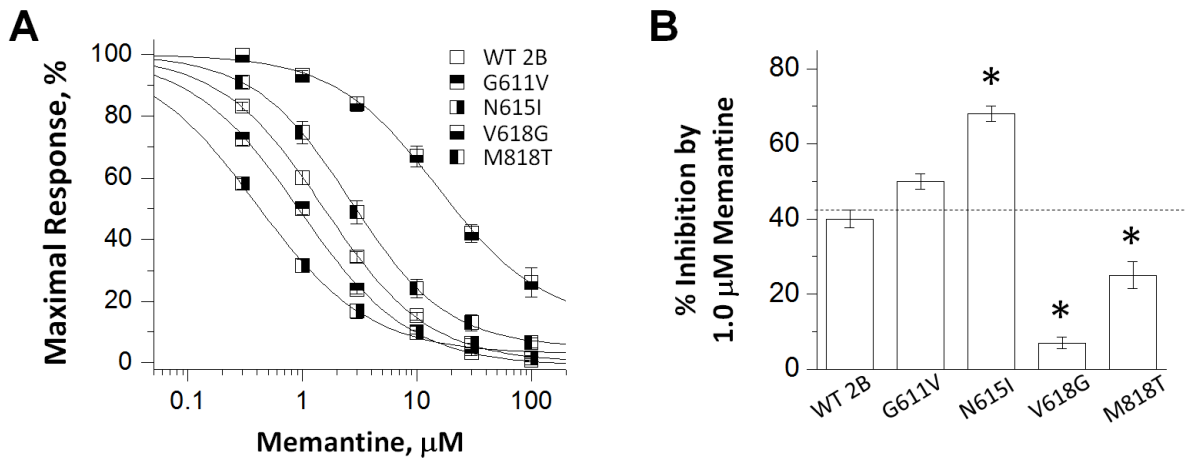
Mean ± SEM

holding at -40 mV

[#] at 1.0 µM memantine

* p<0.05 compared to corresponding wild type (WT); one way ANOVA, Tukey post hoc.

The log of the IC_{50} are normally distributed and were used in all statistical tests.



Supplemental Figure 1. Effect of memantine on gain-of-function GluN2B variants. Composite concentration-response curves for memantine on the current responses (*A*) and percentage inhibition by 1.0 μM (a concentration suggested in brain[9]) memantine (*B*) of four gain-of-function mutants and wild type receptors to maximal effective concentration (100 μM) of glutamate and glycine determined by TEVC recordings from oocytes. *p<0.05, one way ANOVA, Tukey post hoc.

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